# EXPLOITING LIPID METABOLISM BY HSV-1: A CHALLENGE TO RETHINK NEW THERAPIES FOR ALZHEIMER'S DISEASE

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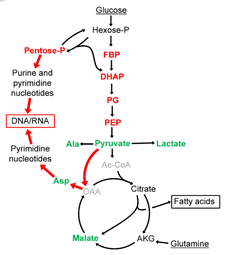
## ABSTRACT

Herpes simplex virus-1 (HSV-1) establishes a life-long latent infection and can enter the brain via retrograde axonal transport. Recurrent reactivation of HSV-1 may lead to neurodegenerative disorders, including Alzheimer's disease (AD), although the underlying mechanisms have not been fully elucidated yet. Lipids constitute the bulk of the brain dry mass and alteration of lipid metabolism is a key component in AD. Considering that the mechanisms for remodeling of metabolism by HSV-1 are still poorly understood, we aim at dissecting the host metabolic pathways modulated by infection in a neuronal-like cell line to identify pathways that might be targeted to prevent AD. Specifically, we found an increase in both de novo synthesis and lipid storage following HSV-1 infection. In addition, anti-AD compounds targeting lipid metabolism (e.g. CMS121, C75) impaired HSV-1 infectivity. Overall, our data unveil new aspects of HSV-1-AD interplay and uncover new potential targets to rethink new possible therapies.

### Effects of HCMV and HSV-1 on central carbon metabolism




Summary of major metabolite concentration and flux changes in response to HSV-1 infection of growth arrested fibroblasts. Arrow colors denote flux changes and font colors denote metabolite level changes relative to the mock-treated control (red-increased, green-decreased, grey-not detected).

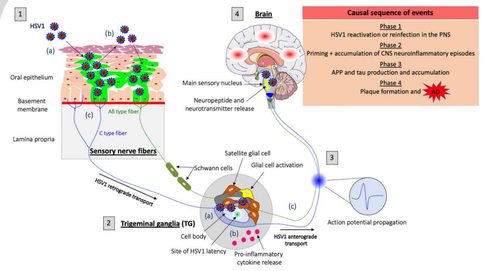
*[Vastag L et al Plos Path 2011]*

## AIM OF THE PROJECT

**Could anti-AD drugs modulate HSV-1 replication and lipid metabolism?**

## BACKGROUND

### HSV-1 as a trigger for AD



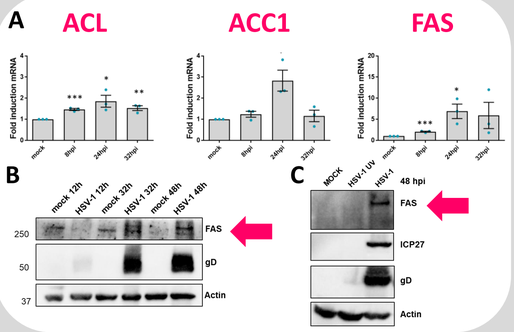

**(1)** HSV-1 replicates in the oral mucosa epithelial cells; **(2)** HSV-1 initiates a productive infection in TG neurons; **(3)** Synaptic transmission of the inflammatory response accompanied by the release of neuropeptides, neurotransmitters and the propagation of specific action potentials from the TG to the main sensory nucleus located in the brainstem;

**(4)** Priming of the brain to a similar inflammatory state following transduction of inflammatory stimuli from the PNS. HSV-1 reactivation or new primary infection of the PNS will thus enhance inflammatory responses in the brain. These events are likely to trigger the production of APP and tau proteins in the brain and repeated inflammation will lead to the accumulation of these proteins and the development of Aβ plaques that cause AD.

*[Laval K & Enquist LW Front Neurol 2021]*

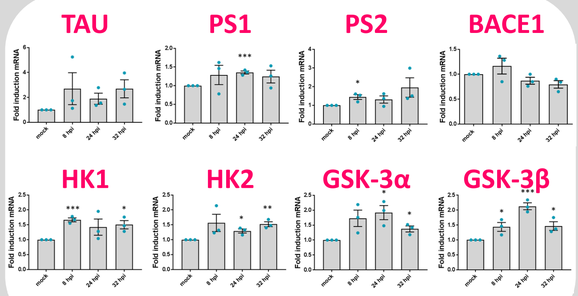
## RESULTS

### HSV-1 induces lipogenic enzymes



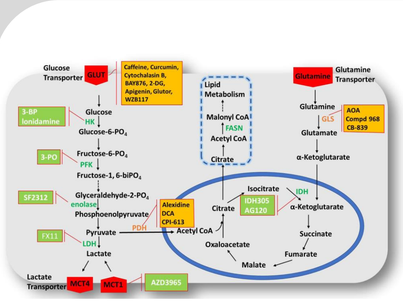

SH-SY5Y cells were infected with HSV-1 (MOI 1). **(A)** At 8, 24, and 32 hpi, total RNA was isolated and subjected to RT-qPCR to measure mRNA expression levels of the lipogenic enzymes ATP citrate lyase (ACL), Acetyl-CoA carboxylase (ACC1), and Fatty acid synthase (FAS). Values were normalized to the housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene mRNA and plotted as fold induction relative to mock-infected cells (set at 1). The results were expressed as the mean ± standard error of the mean (SEM) of three independent experiments (*** P <0.001; ** P <0.01; *, P <0.05; unpaired t test) for the comparison between mock- and infected cells. **(B)** Western blot analysis of protein lysates from mock- or infected cells using the indicated antibodies. A representative blot is shown. **(C)** SH-SY5Y cells were infected with HSV-1 or HSV-1 UV (MOI 1). Western blot analysis of protein lysates from mock- or infected cells using the indicated antibodies. A representative blot is shown.

### Modulation of AD-related genes by HSV-1

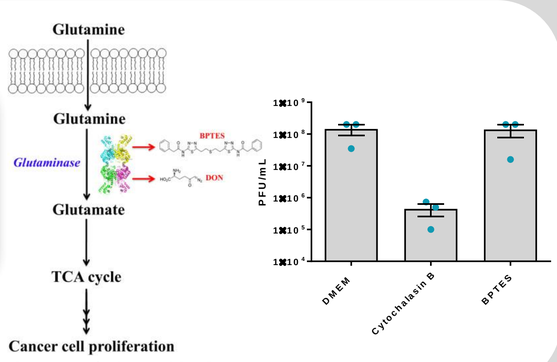



SH-SY5Y cells were infected with HSV-1 (MOI 1). At 8, 24, and 32 hpi, total RNA was isolated and subjected to RT-qPCR to measure mRNA expression levels of the AD-related genes: TAU, presenllin-1 (PS1), presenllin-2 (PS2), β-site APP cleaving enzyme 1 (BACE1), Hexokinase 1 (HK1), Hexokinase 2 (HK2), glycogen synthase kinase 3α (GSK-3α), glycogen synthase kinase 3β (GSK-3β). Values were normalized to the housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene mRNA and plotted as fold induction relative to mock-infected cells (set at 1). The results were expressed as the mean ± standard error of the mean (SEM) of three independent experiments (*** P <0.001; ** P <0.01; *, P <0.05; unpaired t test) for the comparison between mock- and infected cells.

### Glucose is the main source of HSV-1 induced lipogenesis




*[Tilekar K et al Biochim Biophys Acta 2020]*




*[Amin Sa et J Mole Struct 2017]*

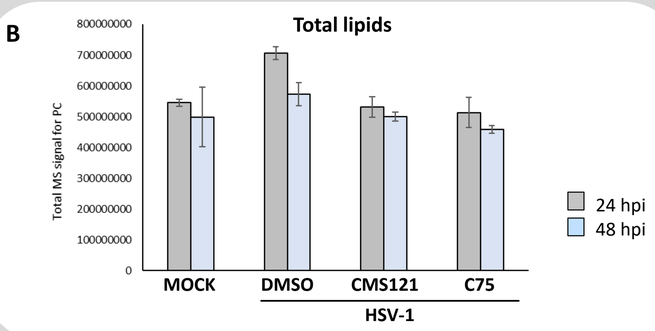
SH-SY5Y cells were infected with HSV-1 (MOI 1) and treated with a non-toxic concentration of Cytochalasin B or BPTES (5 µM). The extent of HSV-1 replication was assessed by titrating the infectivity of supernatants and cell-associated viruses by standard plaque assay. The bars show means and SEMs from three independent experiments (unpaired t-test).

### HSV-1 induced lipogenesis

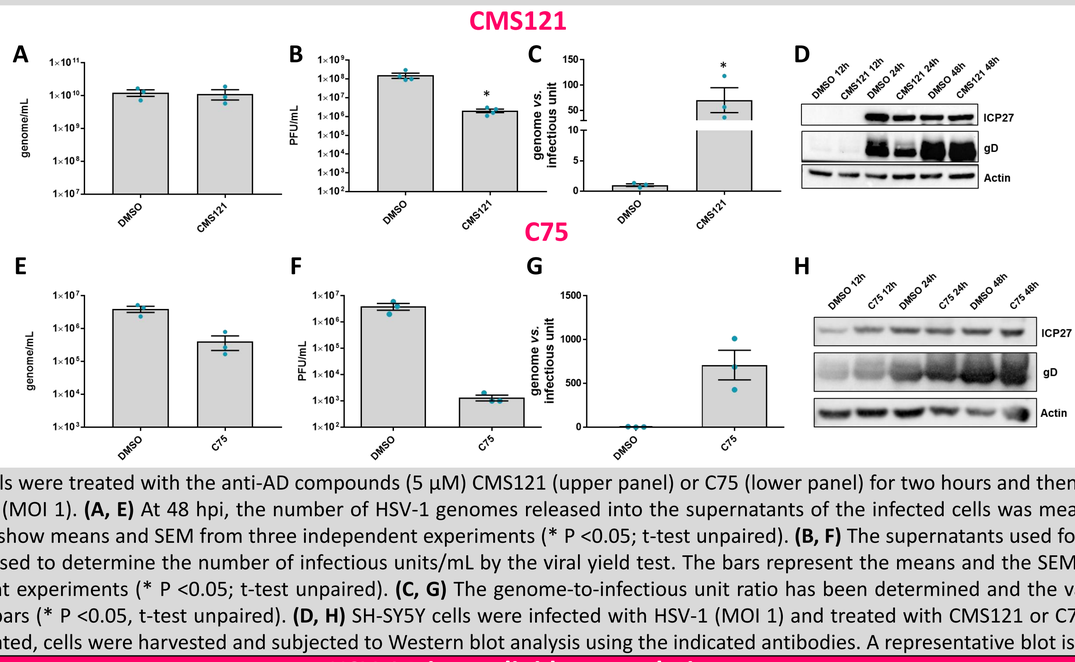
**A**

| LIPID NAME | HSV-1/MOCK 24h FC | HSV-1/MOCK 48h FC | CMS121/DMSO 24h FC | CMS121/DMSO 48h FC | C75/DMSO 24h FC | C75/DMSO 48h FC |
|---|---|---|---|---|---|---|
| EtherPC_30:0 | 1,08 | 1,18 | 1,37 | 1,35 | 1,27 | 1,08 |
| EtherPC_30:1 iso 1 | 1,24 | 1,01 | 1,19 | 1,25 | 1,89 | 1,45 |
| EtherPC_30:1 iso 2 | 1,04 | 1,00 | 1,01 | 1,15 | 0,81 | 0,77 |
| EtherPC_32:0 | 1,12 | 1,41 | 0,99 | 0,99 | 1,02 | 1,05 |
| EtherPC_34:0 | 1,23 | 1,52 | 1,18 | 1,19 | 0,91 | 1,21 |
| EtherPC_34:1 | 1,06 | 1,06 | 1,12 | 1,23 | 1,07 | 0,95 |
| EtherPC_34:2 iso 1 | 0,92 | 1,10 | 0,87 | 1,02 | 1,26 | 0,89 |
| EtherPC_34:2 iso 2 | 1,18 | 1,09 | 0,90 | 1,03 | 0,64 | 0,59 |
| EtherPC_36:1 | 1,28 | 1,62 | 1,61 | 1,65 | 0,85 | 0,77 |
| EtherPC_36:2 iso 1 | 1,08 | 1,13 | 0,84 | 0,98 | 1,09 | 0,94 |
| EtherPC_36:2 iso 2 | 1,12 | 1,17 | 0,87 | 1,00 | 0,66 | 0,59 |
| EtherPC_36:3 iso 1 | 1,06 | 1,19 | 0,96 | 0,84 | 1,47 | 1,31 |
| EtherPC_36:3 iso 2 | 1,13 | 1,08 | 0,85 | 0,92 | 0,69 | 0,59 |
| EtherPC_38:2 | 1,12 | 1,32 | 1,12 | 1,11 | 0,97 | 0,93 |
| EtherPC_38:3 | 1,02 | 1,23 | 0,95 | 0,91 | 1,49 | 1,42 |
| EtherPC_38:4 iso 1 | 1,15 | 1,12 | 0,82 | 0,90 | 1,31 | 1,39 |
| EtherPC_38:4 iso 2 | 1,02 | 1,14 | 0,98 | 0,97 | 1,05 | 0,97 |
| EtherPC_38:5 | 1,06 | 1,20 | 0,97 | 0,95 | 1,38 | 1,24 |
| EtherPC_40:3 | 1,20 | 1,57 | 1,12 | 1,11 | 1,21 | 1,27 |
| EtherPC_40:4 iso 1 | 0,95 | 1,00 | 0,86 | 0,91 | 1,61 | 1,44 |
| EtherPC_40:4 iso 2 | 1,03 | 1,19 | 1,03 | 1,02 | 1,22 | 1,17 |
| EtherPC_40:4 iso 3 | 0,90 | 1,03 | 0,59 | 0,69 | 0,92 | 0,89 |
| LPC_18:1 | 0,54 | 1,07 | 2,02 | 1,25 | 1,26 | 0,83 |
| PC_28:0 | 0,91 | 0,71 | 1,03 | 1,06 | 1,17 | 1,32 |
| PC_28:1 iso 2 | 0,84 | 0,36 | 0,70 | 0,87 | 1,90 | 1,97 |
| PC_30:0_14:0_16:0 | 1,00 | 1,00 | 0,90 | 0,95 | 0,99 | 1,11 |
| PC_30:1 iso 3 | 1,03 | 0,89 | 0,80 | 0,92 | 1,32 | 1,31 |
| PC_31:0_15:0_16:0 | 1,07 | 1,07 | 0,80 | 0,96 | 0,98 | 1,08 |
| PC_32:0_16:0_16:0 | 0,92 | 1,15 | 0,78 | 0,86 | 1,02 | 1,17 |
| PC_32:1 iso 1 | 0,94 | 0,71 | 1,02 | 1,10 | 0,99 | 1,10 |
| PC_32:1 iso 2 | 1,14 | 0,94 | 0,75 | 0,84 | 0,93 | 0,88 |
| PC_32:1 iso 3 | 1,16 | 1,11 | 0,80 | 0,92 | 1,00 | 1,00 |
| PC_32:2 iso 3 | 0,91 | 0,76 | 0,78 | 0,84 | 1,52 | 1,65 |
| PC_33:0_16:0_17:0 | 1,03 | 1,28 | 0,94 | 1,05 | 1,24 | 1,15 |
| PC_33:1 iso 1 | 1,11 | 0,99 | 0,97 | 1,10 | 1,02 | 1,12 |
| PC_33:1 iso 2 | 1,10 | 0,96 | 0,79 | 0,91 | 1,06 | 1,20 |
| PC_34:1_16:0_18:1 | 0,92 | 0,94 | 1,11 | 1,10 | 1,07 | 1,03 |
| PC_34:3 iso 1 | 0,80 | 0,62 | 0,62 | 0,66 | 1,34 | 1,31 |
| PC_34:3 iso 2 | 0,73 | 0,52 | 0,76 | 0,80 | 1,59 | 1,88 |
| PC_35:2_iso 1 | 1,01 | 0,93 | 1,01 | 1,06 | 1,04 | 1,11 |
| PC_35:2_iso 2 | 1,06 | 0,92 | 0,85 | 0,76 | 1,03 | 1,06 |
| PC_36:1_18:0_18:1 | 1,23 | 1,26 | 1,09 | 1,32 | 0,78 | 0,79 |
| PC_36:2 | 1,01 | 0,89 | 1,00 | 1,10 | 0,91 | 0,87 |
| PC_36:3 iso 1 | 0,91 | 0,69 | 0,81 | 0,87 | 1,18 | 1,12 |
| PC_36:3 iso 2 | 1,02 | 0,77 | 0,70 | 0,78 | 0,91 | 0,94 |
| PC_36:4 | 1,16 | 1,06 | 0,78 | 0,55 | 0,95 | 0,86 |
| PC_38:1 | 1,16 | 1,31 | 1,04 | 1,20 | 0,69 | 0,69 |
| PC_38:2 | 1,13 | 1,07 | 0,91 | 1,08 | 0,68 | 0,71 |
| PC_38:3 iso 1 | 0,98 | 0,84 | 0,88 | 1,00 | 0,87 | 0,87 |
| PC_38:3 iso 2 | 1,05 | 0,84 | 0,64 | 0,72 | 0,91 | 0,97 |
| PC_38:3 iso 3 | 1,21 | 1,16 | 0,75 | 0,87 | 1,05 | 1,15 |
| PC_38:4 iso 1 | 0,81 | 0,59 | 0,64 | 0,74 | 1,12 | 1,03 |
| PC_38:4 iso 2 | 1,15 | 1,14 | 0,69 | 0,79 | 0,82 | 0,87 |
| PC_38:5 | 0,98 | 0,87 | 0,69 | 0,73 | 0,86 | 0,80 |
| PC_38:6 | 1,09 | 0,91 | 0,62 | 0,62 | 0,81 | 0,90 |
| PC_40:3 | 1,16 | 1,28 | 0,69 | 0,78 | 0,93 | 0,98 |
| PC_40:4 iso 1 | 0,84 | 0,70 | 0,60 | 0,70 | 0,96 | 1,01 |
| PC_40:4 iso 2 | 1,09 | 1,07 | 0,66 | 0,78 | 0,80 | 0,87 |
| PC_40:5 iso 1 | 0,85 | 0,74 | 0,63 | 0,70 | 0,99 | 1,03 |
| PC_40:5 iso 2 | 1,09 | 1,02 | 0,66 | 0,71 | 0,86 | 0,93 |
| PC_40:6_18:1_22:5 | 0,94 | 0,84 | 0,62 | 0,72 | 0,85 | 0,92 |
| PC_40:7_18:1_22:6 | 1,01 | 0,89 | 0,58 | 0,62 | 0,86 | 0,81 |
| PC_42:4 | 0,86 | 0,76 | 0,60 | 0,75 | 0,81 | 0,85 |

Lipidomic comparison of SH-SY5Y cells infected with HSV-1. **(A)** SH-SY5Y cells were infected with HSV-1 or left uninfected (MOCK) or treated with the indicated compounds or vehicle (DMSO), and then infected with HSV-1 (MOI of 1) and maintained in serum-free medium. At 24 or 48 hpi, total lipids from cells were extracted and analyzed by LC-MS/MS. The fold changes of lipid species peak area are visualized as a heat map showing the levels of upregulated (red, fold change > 1) and downregulated (blue, fold change < 1) glycerophospholipids. PC, glycerophosphocholine. n = 2 independent determinations. **(B)** Histogram reporting the total MS signal for PC in the different conditions.

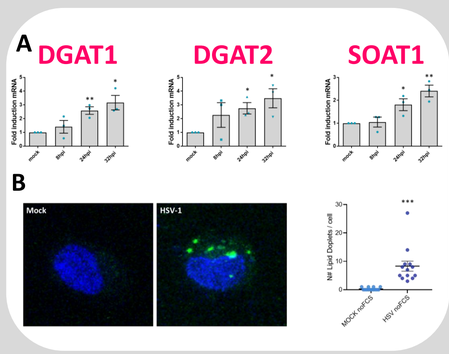



### CMS121 and C75 reduce the infectivity of HSV-1 viral particles




SH-SY5Y cells were treated with the anti-AD compounds (5 µM) CMS121 (upper panel) or C75 (lower panel) for two hours and then infected with HSV-1 (MOI 1). **(A, E)** At 48 hpi, the number of HSV-1 genomes released into the supernatants of the infected cells was measured by qPCR (bars show means and SEM from three independent experiments (* P <0.05; t-test unpaired). **(B, F)** The supernatants used for panel A or E were used to determine the number of infectious units/mL by the viral yield test. The bars represent the means and the SEM of three independent experiments (* P <0.05; t-test unpaired). **(C, G)** The genome-to-infectious unit ratio has been determined and the values are above the bars (* P <0.05, t-test unpaired). **(D, H)** SH-SY5Y cells were infected with HSV-1 (MOI 1) and treated with CMS121 or C75. At the times indicated, cells were harvested and subjected to Western blot analysis using the indicated antibodies. A representative blot is shown.

### HSV-1 triggers lipid accumulation




SH-SY5Y cells were infected with HSV-1 (MOI 1). **(A)** At 8, 24, and 32 hpi, total RNA was isolated and subjected to RT-qPCR to measure mRNA expression levels of enzymes involved in lipid accumulation: diacylglycerol O-acyltransferase 1 (DGAT1), diacylglycerol O-acyltransferase 1 (DGAT2), sterol O-acyl transferase 1 (SOAT1). Values were normalized to the housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene mRNA and plotted as fold induction relative to mock-infected cells (set at 1). The results were expressed as the mean ± standard error of the mean (SEM) of three independent experiments (*** P <0.001; ** P <0.01; *, P <0.05; unpaired t test) for the comparison between mock- and infected cells. **(B)** SH-SY5Y cells were infected with HSV-1 (MOI 1) for 48 h. All cells were stained BioTracker 488 Green Lipid Droplets Dye to visualise LDs (green) and DAPI to visualise the cell nuclei (blue). LD numbers were analysed using ImageJ analysis software. The results were expressed as the mean ± standard error of the mean (SEM) of three independent experiments (*** P <0.001; unpaired t test) for the comparison between mock- and infected cells.

## CONCLUSIONS

- HSV-1 infection increases de novo fatty acid synthesis through transactivation of lipogenic enzymes.
- HSV-1 infection increased lipid storage.
- The anti-AD compounds CMS121 and C75 act inhibit HSV-1 replication.
- Some genes involved in AD are deregulated following HSV-1 infection.

## CONTACTS

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